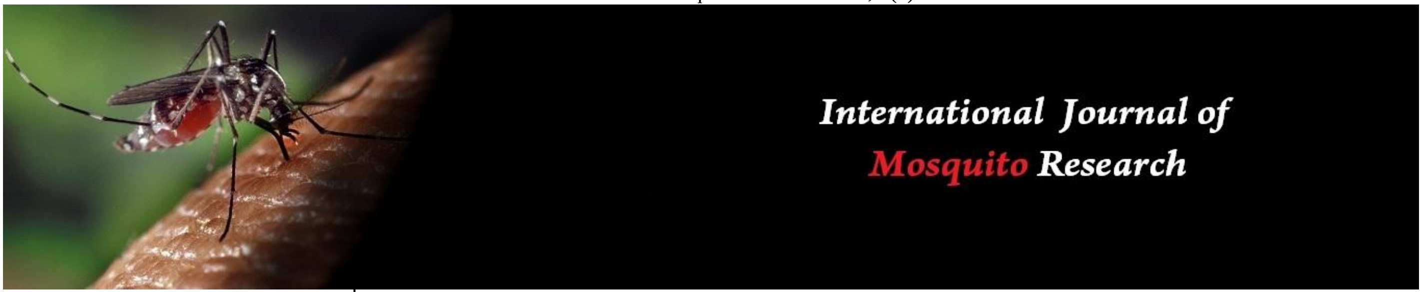

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Malaria vector transmission dynamics in Bandiagara, a potential site for vaccine candidate trial in Mali

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Abstract
Experience from other parts of the world suggested that dropping malaria infection to zero might be possible only with the help of a vaccine. In this dynamic, the National Institutes of Health of USA decided to explore the possibility of implementing a trial of malaria vaccine candidate in Bandiagara (Mali). The study aimed to characterizing vector composition, seasonal variations, human and host contact, infection rate and the entomological inoculation rate (EIR) variations before vaccine trial implementation. *An. gambiae* s.l. and *An. funestus* were found to be the main malaria vectors in the area. Transmission was dynamic and seasonal, but very intense during the rainy season. The peak of EIR occurred at the end of the rainy season and was different between strata. The intensity and seasonality of malaria transmission in the area could justify the suitability of the site for a potential malaria vaccine trial.

Keywords: Malaria transmission, vector biology, *An. gambiae* sl., *An. funestus*, EIR, Mali

1. Introduction
Malaria is the first public health problem in sub-Saharan Africa. This disease is acquired by the infection of *Plasmodium* to human by the bite of female *Anopheles*. According to WHO, 93% of malaria deaths are occurring inside the 219 million of cases in Sub Saharan African countries [1]. Many studies have shown the resistance of the most dangerous species *Plasmodium falciparum,* to chloroquine treatment [2-4].
In Mali several studies were done to assemble the epidemiological evidence base on more targeted approach to malaria control in the country. A consistent data of survey from several locations were published [5] and used as reference of malaria epidemiology for national policy documents since 1993. Other investigation have been done in the south of Mali, looking at the impact of chemoprophylaxis on malaria seasonality [6-8]. An experimental study in two villages of hiperendemic malaria by the use of impregnated curtains have shown significant reduction of the entomological inoculation rate in this country [9]. When surveys showed more than 30% of parasite resistance levels to chloroquine [10], a very important political decision was made by the government. So taxes on the regular bed nets and insecticide treated nets was abolished in the country [11]. Recently, efforts including scientific studies, medical assistance and political engagement, showed important progress on the reduction of malaria cases, but also the need for complementary strategies as advised by WHO so far. The necessity of integrated approaches against both vectors and parasites are critical for effective malaria control [12]. The elimination of malaria cases in endemic villages might be possible only with the help of vaccine. Nonetheless, the spread of drug resistant parasites and insecticide resistant mosquitoes [13] has caused much interest in the use of biological tools, computer prediction [14-18] or vaccines to control malaria. In this dynamic, the National Institutes of Health of United States of America and the Malaria Research and Training Center (MRTC) in Mali, decided to explore the possibility of implementing a trial of malaria vaccine candidate in Bandigara city, in Mali. That requires detailed information on the local epidemiology of malaria and the distribution of risk groups. An ideal indicator of malaria risk is the entomological inoculation rate (EIR), an index that relates both the human-biting activity of the *Anopheles* vectors and

the risk to humans of malaria infection [19].

Althrough *An. gambiae* s.l and *An. funestus* have been identified as the main malaria vectors across Mali [20-25], there was no detailed information on malaria transmission in the village of Bandiagara by theses vectors. The level of malaria transmission can be estimated from many parameters which can be entomological [26], parasitological [27, 28], clinical [28, 29] or immunological [30, 31]. The most common entomological method used is the estimation of the entomological inoculation rate (EIR) by combining an estimated number of mosquito bites per human per day [32], which itself is calculated from human biting rate, with information on the prevalence of Plasmodium sporozoites infection rates [33] of vectors.

The local and seasonal EIR estimates can then be used to inform and focus malaria control strategies. The current study aimed at characterizing the site for vaccine trials by identifying vectors composition, their seasonal variations, the human and host contact as well as infection index and the dynamic of the entomological inoculation rates (EIR).

2. Material and Method

2.1 Study site

This study was performed in Bandiagara city located at about 665 km North-East from Bamako district, the capital city of Mali. With 10 000 inhabitants, Bandiagara is located in the Sahelian zone between the 200 and 700 mm isohyets. The rectangular mud-brick, mud-roof houses of the Dogon and Fulani ethnic group respectively (70% and 25% of the population) are clustered together in adjacent compounds. The community grows primarily millet, sorghum, maize (corn), and peanuts during the rainy season (July to September). Cattle, sheep, goats, and chickens are bred by most families. The rains fill two large ponds and numerous small puddles, but usually, all surface waters dry by November except the YAME River. From November until May, rainfall is altogether absent or negligible (total precipitation < 30 mm). After the harvest (October to November), the fields surrounding the city lay bare. The only main water body available for mosquitoes breeding sites is the YAME River. Annual precipitation is approximately 500 mm. The Bandiagara region is characterized by a short rainy season of about three months (from July to September). The vegetation is composed by species like *Acacia albida*, *Vittelaria paradoxa*, *Euphorbia balsamifera*, *Euphorbia sudanica*. The city is composed by 9 neighborhoods in total, one for administration workers and the 8 other for the main population. Based on the particular geographical characteristics of each area of interest for a good coverage, the study site was divided into three (3) strata (figure 1):

- Stratum 1: Composed of neighborhoods I, V, VI and VII. It is crossed from East to South-West by YAME River, the main water body of Bandiagara almost permanent. It is the largest stratum.
- Stratum 2: This is the administrative residence proper. It is at altitude compared to the rest of the city. The urbanization of this stratum gives it a particular aspect; individual concessions, vast and isolated, large streets with a general electrification.
- Stratum 3: Consisting of Quarters II, III, IV and VIII. It is bordered by temporary water streams in the rainy season. Compounds are agglomerated as in some parts of the first stratum.

2.2 Sample collection

A longitudinal study with regular monthly cross sectional visits in the second half of each month was undertaken during the period from June to December 2002. The study was conducted to understand the biology of the vector and malaria transmission indices based on the assessment of human biting rate, anthropophilic rate, sporozoite infection rate as well as the entomological inoculation rate (EIR). During 14 days of field work in each month, two collections methods were used: Pyrethrum spray-catch during day time and the human landing catch (HLC) at night.

2.2.1 Pyrethrum spray-catch (PSC): Spray-catches sessions were organized with 30 to 45 houses investigated per session depending on the stratum. In total, 180 houses were visited per month respecting the following distribution: Stratum 1 = 90 houses; Stratum 2 = 30 houses and in Stratum 3: 60 houses. Mosquitoes from this collection method were first morphologically identified, sorted per sex, per species and per gonotrophic status with Id number. Later these mosquitoes were used to determine malaria vectors composition, frequencies determination and infection rate using PCR and/ or ELISA method.

2.2.2 Human landing catch (HLC): The HLC was performed twice in each stratum per visit. A total of 6 sessions of HLC using 16 collectors were done. At each collection site a group of 4 persons is operating outside and 4 other people inside human sleeping rooms from 06:00pm to 12:00pm, exchanging every two hours. For efficiency, a second set of same number of collectors were replacing the first group from 00:00am to 6 am early the next morning. Mosquitoes from HLC collection method were morphologically identified and recorded by species and gonotrophic status with individual Id number. Each unfed female *Anopheles* species was dissected to check the ovaries for nulliparty (never lay eggs) or multiparity (Laid eggs at least once) by Detinova method [34]. This can show if most of the collected females are newly emerged from breeding site, with no biting experience yet, or if they already had single of multiple blood meals and then become susceptible to carry/develop *Plasmodium* infection. Mosquitoes from this collection method were used to determine the human biting rate, infection rate and the entomological inoculation rate (EIR).

2.3 Ethical issue

Prior to the study, the research protocol was defended and approved by the national ethical comity. After that, it was also clearly explained to the administration as well as to traditional authorities of Bandiagara city for their approval. Finally, the acceptance of all house owner was individually obtained before investigation in the particular house. The collectors received money for time lost compensation and treatment when they are sick. Unique individual Id was given to each house involved in the study. All individual data was confidentially treated. Only global result was showed, reported to the community and now proposed for publication.

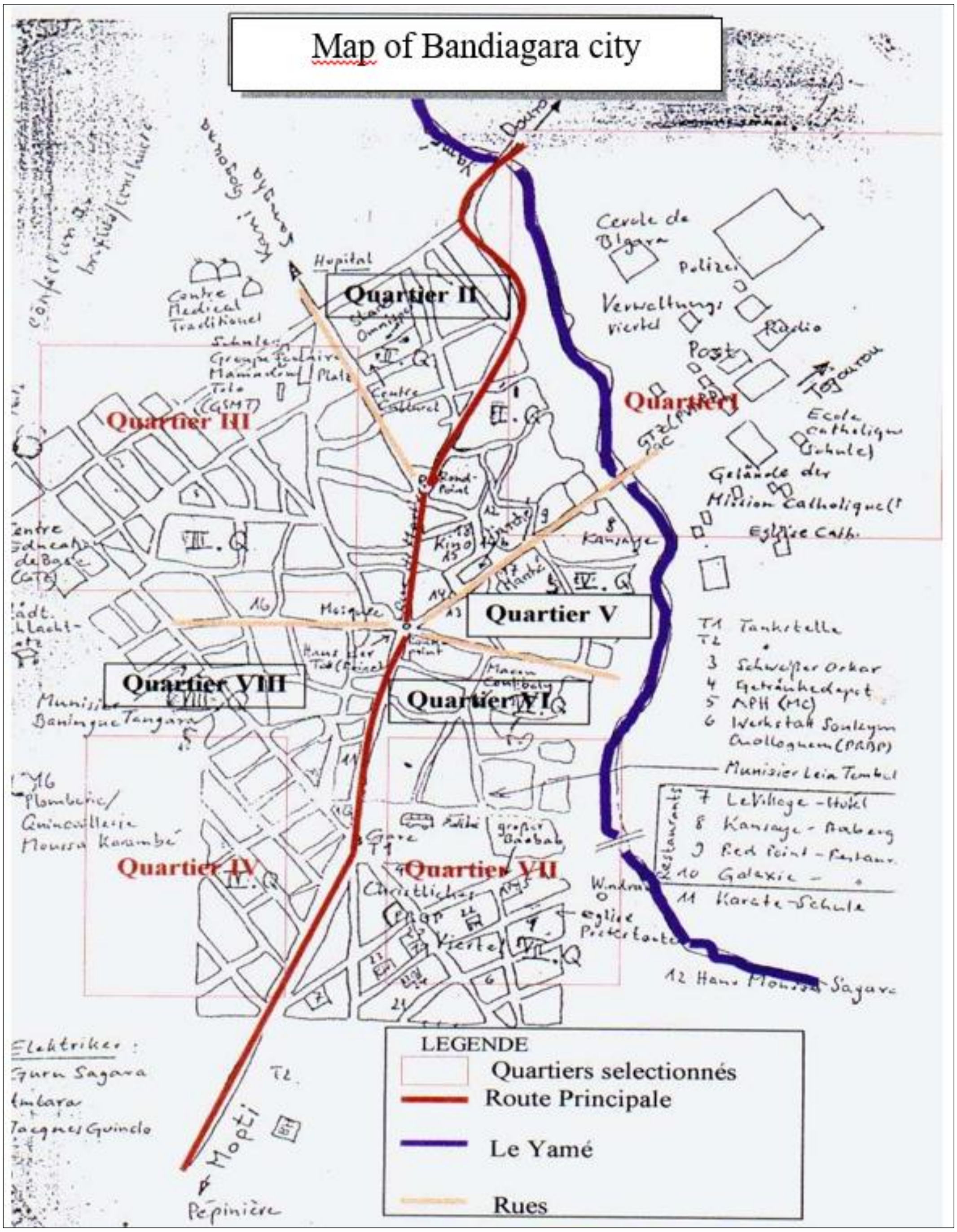

Fig 1: Bandiagara city map (Source, Bandiagara Malaria Project Entomology team, 2000)

3. Results
3.1 Vector composition
3.1.1 Monthly variations of the relative frequencies of *An. gambiae* s.l and *An. funestus*

Table 1: Monthly variations of the relative frequencies of *An. gambiae* s.l and *An. funestus* collected by spray catch (PSC) from 180 houses each month in Bandiagara from June to December 2002.

Species	*An.gambiae s.l*		*An. funestus*		Total
Months	N	%	N	%	
June	103	100.0	0	0.0	103
July	624	100.0	0	0.0	624
August	2468	100.0	0	0.0	2468
September	2509	100.0	0	0.0	2509
October	1210	99.8	3	0.2	1213
November	816	99.5	4	0.5	820
December	182	100.0	0	0.0	182
Total	7912	99.9	7	0.1	7919

Malaria vectors abundance is increasing from the beginning of the rainy season (June) and declines at the end of wet season. The peak of vectors frequencies occurred in August to September with respectively 2468 and 2509 anopheles collected (table 1). Data showed that *An. gambiae* s.l and *An. funestus* are the only malaria vectors in Bandiagara, but with very high proportion (99.9%, n = 7919) of *An. gambiae* s.l from the total population all year round (table 1). *An. funestus* was found only at the cold dry season (October-November). The lowest frequencies was observed in December (182) and June (103), (table 1).

3.1.2 Variations of the relative frequencies of *An. gambiae s.s.* and *An. arabiensis* per month and per stratum.

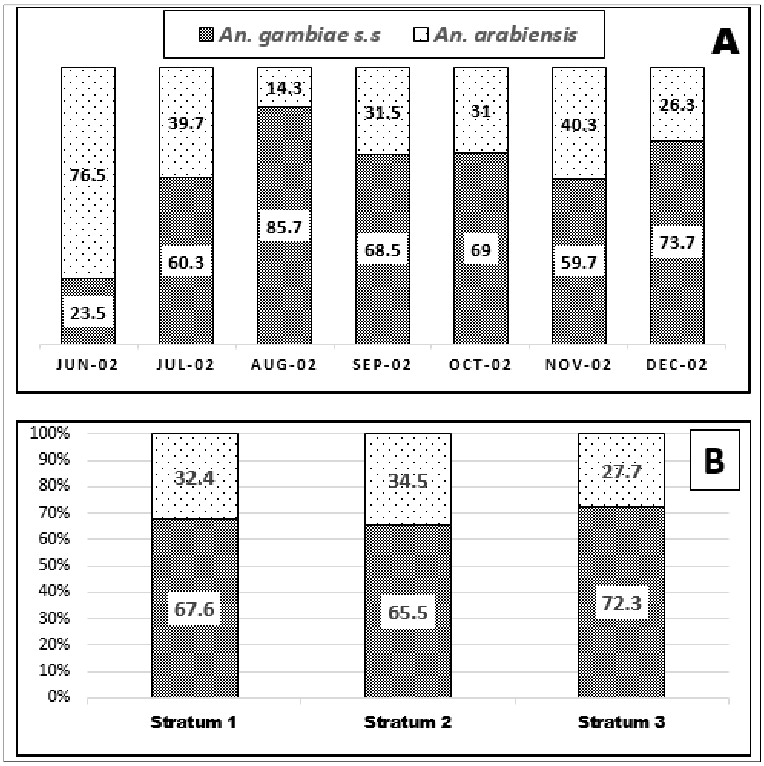

Fig 2: Variations of the relative frequency (%) distribution per month (A) and per stratum (B) of *An. gambiae s.s.* and *An. arabiensis* in Bandiagara from June to December 2002.

After *An. gambiae* s.l, vectors populations identification by PCR, data have shown that they were composed by *An. gambiae s.s* and *An. arabiensis* (figure 2 A and B). For the whole study period relative frequency of *An. gambiae s.s*, 68.3% (n = 2164) was significantly higher than that of *An. arabiensis* (31.7%) except in June where 76.5 of the sample were *An. arabiensis* and 23.5% *An. gambiae s.s*. There was significant monthly variations of the relative frequencies of *An. gambiae s.s* and *An. arabiensis*; (Chi-square=169.40, df=6, $P<10^{-8}$), (figure 2 A). Also, the relative frequencies of *An. gambiae s.s.* and *An. arabiensis* showed significant variations between the three strata (Chi-square = 7.79; ddl =2; P = 0.020; figure 2 B).

3.2. Malaria vectors transmission assessment

3.2.1. Monthly human biting rate for *An. gambiae* s.l

Table 2: Monthly human biting rate (number of mosquito bites per man per month) for *An. gambiae* s.l collected by Human Landing Catch (HLC) in Bandiagara from June to December 2002.

	Human landing Catch (HLC)		
Months	Nb. sleepers	Nb. mosq	Bites/man/month (*An.gambiae s.l*)
June	24	24	30.0
July	24	124	155.0
August	24	363	453.8
September	24	288	360.0
October	24	168	210.0
November	24	121	151.2
December	24	29	36.3
Total/mean	*168*	*1117*	*199.5*

The average monthly man-biting rate during the study period was 199.5 bites per man per month for *An. gambiae* s.l. It showed its highest value in August (453.8 bites per man per month) and the lowest in June (30.0, table 2). The man bating rate increased from June (30 bites per man) to August and decreased from August to December (36.3 bites per man).

Table 3: Mean monthly man biting rate for *An. gambiae* s.l (number of mosquito bites per man per month) collected by HLC in the different strata from June to December 2002.

Human landing Catch (HLC)			
Strata	**Collectors**	**Nb. mosq**	**Man biting rate**
I	56	162	86.8
II	56	494	264.6
III	56	461	247.0
Total	168	1117	199.5

The average monthly man-biting rate from human landing catch at the night showed its highest value in stratum II (264.6 bites per man per month) and its lowest value in the stratum I (86.8), (table 3). The cumulative monthly man biting rate over the study period was 607.2 bites in stratum I, in stratum II it was 1852.2 bites and 1728.3 bites in stratum III**.**

3.2.2 Parous rates, daily survival rates, anthropophilic rates, and infection rates for *An. gambiae* s.l.

Table 4: Parous rates, daily survival rates, anthropophilic rates, and infection rates for *An. gambiae* s.l during each month and per stratum, in Bandiagara from June to December 2002

Entomological Parameters					
Parous rates and daily survival of *An. gambiae s.l.*					
	Months	**#Mosq**	**Parous**	**%**	**Daily survival rate %**
Monthly variation	June	10	9	90.0	95.0
	July	74	67	90.5	95.2
	August	205	162	79.0	89.9
	September	209	139	66.5	81.2
	October	115	93	80.9	90.0
	November	98	82	83.7	91.5
	December	16	16	100.0	100.0
	Total	727	568	78.1	88.4
	Strata	**#Mosq**	**Parous**	**%**	**Daily survival rate %**
Variation per stratum	Stratum I	135	97	71.9	84.8
	Stratum II	364	296	81.3	90.2
	Stratum III	228	175	76.8	87.6
	Total	727	568	78.1	88.4
Anthropophilic rate of *An. gambiae s.l.*					
	Months	**#Mosq**	**Positive**	**%**	
Monthly variation	June	35	34	97.	
	July	242	226	93.	
	August	675	597	88.	
	September	924	837	90,6	
	October	432	398	92.	
	November	292	273	94.	
	December	67	67	100.0	
	Total	2667	2432	91.	
	Strata	**Total**	**Positive**	**%**	
Variation per stratum	Stratum I	1297	1203	92.8	
	Stratum II	419	379	90.5	
	Stratum III	951	850	89.4	
	Total	2667	2432	91.2	
Infection rate of *An. gambiae s.l.*					
	Months	**#Mosq**	**Positive**	**%**	
/40411.v. variation	June	103	0	0.0	
	July	624	23	3.7	
	August	2467	54	2.2	
	September	2501	117	4.7	
	October.	1215	63	5.2	
	November	818	37	4.5	
	December	182	8	4.4	
	Total	7910	302	3.8	
	Strata	**Wog**	**Positive**	**%**	
Variation per stratum	Stratum I	3155	151	4.8	
	Stratum II	1797	46	2.6	
	Stratum III	2958	105	3.5	
	Total	7910	302	3.8	

The average parous rate for *An. gambiae* s.l. was 78.1% (n=727), ranging from 66.5% in September to 100.0 in December. The lowest parous rate was obtain in the middle of the rainy season in September. There was a significant difference between the monthly parous rates. (Chi-square =1530.86; df=6; P=0.00002; table 4). The parous rates between the strata were not significantly different (Chi-square = 5.53, df=2, p=0.062, table 4).

The mean daily survival rate for *An. gambiae* s.l. calculated from the monthly parous rate varied from 81.2 % in September to 100% in December for a gonotrophic cycle duration of about 2 days. So, the females daily survival is high when density is low like in June (95%), July (95.5%) and December (100%). Females of *Anopheles* s.l showed high daily survival rate in all strata: 90.2%, 87.6% and 84.8% for stratum II, stratum III and stratum I respectively.

The mean anthropophilic rate was 91.2% (n=2667, ranging from 88.4 in August to 100% in December). There was a significant monthly variation of the anthropophilic rates (Chi-square=18.63; df=6; P=0.004), with in average December (100%) showing higher anthropophilic rates than August (88.4%; table 4). There was no significant variation of the anthropophilic rates between the three strata (Chi-square =8.53; df=2; P=0.014; table 4). The anthropophilic rate in the different strata was 92.8, 90.5 and 89.4 for stratum I, stratum II and stratum respectively.

The average infection rate of *An. gambiae* s.l. was 3.8% (n=7902). There were significant monthly variations of the CSP infection rates (Chi-square=30.11; df =5; P< 10^{-3}, excluding June; table 4). The mean infection rate was 3.8% (n=7910). Mosquitoes begin to be infected in July and remain infected up to December with significant variations from the beginning of infection season to the end. There were significant differences of the overall CSP infection rates among the three strata **(**Chi-square =15.86; df=2; P=0.00035). The CSP infection rate in stratum I (4.8%, n=3166) was significantly higher than those of strata II (2.6%, n=1797) and III (3.5%, n =2958; Table 4).

3.2.3 Entomological inoculation rates rates (EIR) for *An. gambiae* s.l

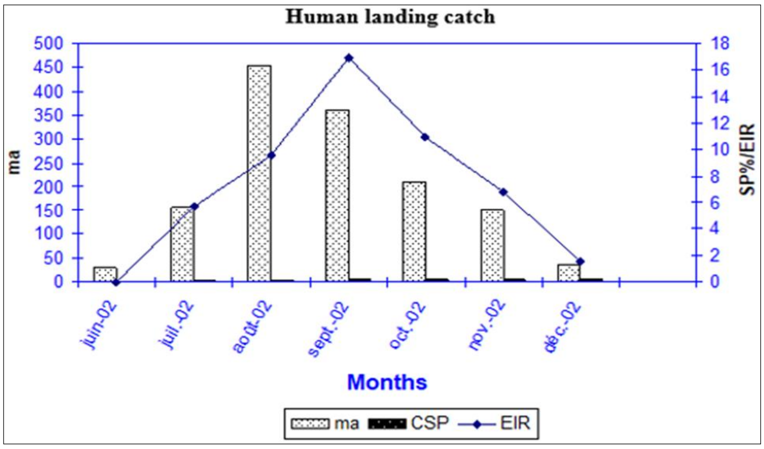

Fig 3: Monthly entomological inoculation rates (EIR) for *An. gambiae* s.l. collected by HLC in Bandiagara from June to December 2002.

The cumulative monthly entomological inoculation rate (EIR) for *An. gambiae s.l.* from night catches for the whole study period was 51.5. The highest value of the EIR was observed in September (16.92 infective bites per man per month; figure 3). From zero in June, the EIR started to increase continually from July, August up to September and then begin to decrease progressively from September to December.

Table 5: Variations of entomological inoculation rates (EIR) for *An. gambiae* s.l, per stratum from HLC at Bandiagara from June to December 2002.

Human landing Catch (HLC)			
Months	ma	CSP	EIR
Stratum I	86.8	0.048	4.2
Stratum II	264.6	0.026	6.9
Stratum III	247.0	0.035	8.6

For the study period, the cumulative EIRs from night catches were higher in stratum III (8.6 infective bites per man per month) followed by stratum II (6.9 infective bites) and stratum I (4.2 infective bites); table 5. The higher rate EIR was obtain in stratum III.

4. Discussion

4.1 Vector composition

Data showed that the *An. gambiae* complex (*An. gambiae* s.l) and *An. funestus* are the main vectors of malaria in Bandiagara city. *An. gambiae* s.l was significantly dominant compared to *An. funestus* which looks to be a secondary vector. This vector composition is very common in West African malaria endemic countries [35- 38]. But other *Anopheles* species can contribute to transmit malaria in addition to *An. gambiae* s.l and *An. funestus* in some nearest countries [39, 40]. Monthly variations showed that the peak of the density on malaria vectors in Bandiagara is occurring during the very wet season from August to September. Within the *Anopheles* complex, *An. gambiae s.s* is predominant compare to *An. arabiensis* year round in all strata. There were also significant monthly variations of the relative frequencies of *An. gambiae s.s* and *An. Arabiensis*. This variation is probably due to the variation of breeding site availability due to rainfall.

4.2 Vectors biting rate

The human biting rate per person and per month is very high during the very wet season (August to September) from and HLC measurements, with very high risk among people sleeping without protection as shown by the data from human landing catch exposure (453.8 bites per month per person in August and 360 bites per month per human in September (table 2). The lowest value of biting rate was found in June. The variations of the biting rate per month and per stratum are related to the vector population densities. As reported by several studies, *An. gambiae* s.l is known to be very anthropophagic (Preference for human blood) [38]. Between the three strata, higher risk of mosquito biting rate was obtained in stratum II and the lowest value in stratum I, from HLC collection methods. This is may be due to the structure and houses agglomeration combined with potential breeding sites availability nearby the stratum II. The factors at risk associated with vector high biting rate have been shown by other studies across the world [41- 44].

4.3 Females *An. gambiae* s.l parous rate and daily survival rate

With up to 78.1% of parous rate and 88.4% daily survival rate in average, that mean the vector population in Bandiagara city is composed by aged females with higher survival. These indicators support the capacity of the females to have several blood meals for eggs production during their life. Assuming that each biting for blood meal is a risk factor of infection, these data can explain somehow why the area is endemic for malaria. This assessment is also true in all three strata (high parous rate > 70%, and high daily survival rate >84%).

4.4 Vector anthropophlic rate and infection rate

Data showed that *An. gambiae* s.l has a strong preference for human blood. At all period of year the females feed on human in all strata with a rate greater than 89%. This is one of the major reason for the necessity to make a barrier between human and the vector for malaria transmission control [44-46]. Mosquito can be infected by feeding on human hosts having *Plasmodium* parasites in their blood. So, in malaria endemic area, the more a female *Anopheles* is susceptible to bite human subject, the more it is susceptible to be infected. In the current study, infection was found with female *An. gambiae* s.l. almost during the whole investigation period with different levels. The infection rate has increased progressively from July (3.7%) to reach a peak in October (5.2%) and declined progressively to 4.4% in December. The peak of infection occurred at the end of the rainy season. That can be explained by the age of the majority of females. Because by October, most of the breeding sites were dried out and the few newly emerged mosquitoes would not be able to dilute the infection in the main female vector populations. The oldest females in the nature have more chance to be in contact with the *Plasmodium*. With an average of 3.8% infection rate per month, the risk for Bandiagara citizen to receive infective bite is high. Mosquitoes from stratum III were most infected (3.5%) than those from stratum I (4.8%) and the stratum II (2.6%). This means that in the same city, the risk to receive infective bites is different from one stratum to the other as well as from one group of people to another, as reported by other authors [42, 47, 48].

4.5 Vector entomological inoculation rates (EIR)

The entomological inoculation rate is related to the biting rate and the vector infection rates Here the peak of monthly entomological inoculation rate calculated from HLC collection method was observed in September with 16.9 infective bites per person and per month. This means that, people living in Bandiagara city with no permanent protection were exposed to a risk of receiving up to 16.92 infective bites within a single month (September). The cumulative monthly entomological inoculation rate (EIR) for *An. gambiae* s.l. is 51.5 from HLC, This showed that any person staying in Bandiagara from June to December can receive more than 50 infective bites in this period. That can explain easily why a same person can develop several malaria episodes in the same rainy season if his protection is not efficient [49]. Comparison of infection risk between strata, showed that people living in stratum III with 8.6 infective bites for the study period were more exposed to malaria compared to those in stratum II (6.9 infective bite) and stratum I (4.2 infective bites). The risk factors associated with arthropod borne diseases [50, -54] such as malaria was reported by several authors in different studies in different countries [47]. But the contribution of local community to facilitate control activities have been showed in some countries [55].

5. Conclusion

This study conducted with monthly cross-sectional surveys from June 2002 to December 2002, showed that *An. gambiae s.l.* and *An. funestus* are the main malaria vectors with high proportion of *An. gambiae* s.l. composed by *An. gambiae s.s* and *An. arabiensis.* Malaria vectors in Bandiagara are highly anthropophilic blood. Vectors were very aggressive in the heavy rainy period from August to September. The infection rates and the entomological inoculation rates were high and optimal at the end of raining season and different from one stratum to another. There was a higher risk of several reinfections of the same person in the same transmission season. The result assessment of the different malaria indices could justify the suitability of the study site for a potential malaria vaccine trial.

6. Acknowledgement

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